APPARATUS AND METHOD FOR IDENTIFICATION OF CRYSTALS BY *IN-SITU* X-RAY DIFFRACTION

BACKGROUND OF THE INVENTION

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) of prior U.S. Provisional Application No. 60/242,034, filed October 19, 2000.

FIELD OF THE INVENTION

The present invention relates generally to crystallography, and in particular to an automated apparatus and method for the high throughput analysis of crystals in their *in-situ* growth environment.

DESCRIPTION OF RELATED ART

X-ray diffraction is a powerful technique for determining the structure of molecules. For a general review of X-ray diffraction, *see* B.E. Warren, *X-ray Diffraction*, Dover, 1990, or Cantor and Schimmel, *Techniques for the Study of Biological Structure and Function*, W.H. Freeman, 1980. In general, the three-dimensional structure of a molecule can be determined by observing the diffraction of a high-intensity beam of X-rays from a crystalline form of the molecule. Typically, a beam of X-rays is passed through a crystalline form of a molecule, whereafter data is collected from the unique diffraction patterns of the crystal. The diffraction data from one or more crystalline forms of the molecule can be used to calculate the three-dimensional structure of the molecule. The quality of the X-ray diffraction and, therefore, the quality of the three-dimensional structure depend on the quality of the crystalline forms of the molecule. Highly ordered, stable crystals tend to generate higher quality X-ray diffraction data.

Unfortunately, crystallization of a molecule is not a trivial task. The conditions required to grow crystals (crystallization conditions) may be dependent on

many variables such as pH, buffer type, buffer concentration, precipitant, precipitant concentration, ionic strength, concentration of the molecule to be crystallized, temperature, and so forth. These crystallization conditions vary from molecule to molecule and must often be determined empirically by trial and error. In these cases, hundreds or thousands of conditions must be explored before a single candidate crystal of a molecule can be observed. For macromolecules, including proteins, crystallization can be prohibitively difficult as concentrated, highly purified solutions of macromolecules are difficult to obtain. Even then, such concentrated purified solutions often have limited stability.

Even if a first crystalline form of a molecule is obtained, there is no guarantee that this crystalline form of the molecule will diffract X-rays sufficiently well to obtain high resolution structural information. Often, the crystals are not of sufficient size or not sufficiently well-ordered to adequately diffract X-rays. Typically, researchers must laboriously optimize crystallization conditions to arrive at a crystal of sufficient quality for high-resolution diffraction of X-rays. Unfortunately, optimization of crystallization conditions is currently performed by similar trial and error techniques that are used to discover the first crystallization conditions.

To further complicate this process, crystallization conditions are not always optimized according to parameters that necessarily lead to improved X-ray diffraction. Typically, crystallization conditions are optimized to yield crystals of larger size and better visual appearance. Unfortunately, the size of a crystal and the visual appearance of a crystal are not well correlated to higher resolution diffraction of X-rays. In other words, a larger crystal does not necessarily diffract X-rays to a higher resolution than a smaller crystal. Similarly, a crystal with a superior visual appearance does not necessarily diffract X-rays to a higher resolution than a crystal with an inferior visual appearance.

Furthermore, initial crystallization experiments often yield tiny aggregates of molecules with an appearance or morphology that is difficult to identify. These particles could be poorly ordered, or amorphous, precipitate that might not be useful for further structural experiments. On the other hand, these particles could be microcrystals that satisfactorily diffract X-rays. Such microcrystals indicate initial

crystallization conditions that could be optimized to yield crystals suitable for X-ray diffraction and data collection. Unfortunately, current crystallization techniques, such as visual inspection of crystals, even with the aid of a light microscope, cannot distinguish between amorphous precipitate and useful microcrystals.

Conventionally, cumbersome methods are used to investigate the actual diffraction quality of a candidate crystal. In a typical method, a candidate crystal is first removed from a crystallization solution. The delicate crystal is then transferred, usually by hand, into a capillary tube , or into cryosolution and into a cryoloop, which is placed in an X-ray beam for observation of the diffraction quality of the crystal. This relocation can easily damage the fragile crystal. What is more, such method can only be used to observe a few candidate crystals at a time.

Accordingly, there is a need in the field of crystallography for improved techniques for the systematic discovery and optimization of ideal crystallization conditions.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a method and apparatus for the identification and optimization of a crystal *in-situ*, *i.e.*, in its crystallization solution. The apparatus and methods can thus be used to assess crystallization conditions without reliance upon visual inspection of a crystal and without removal of a crystal from its *in-situ* growth environment. Since potential crystals do not need to be removed from their *in-situ* growth environment, crystallization conditions can be inspected for diffracting material several times during the crystal growth period. In addition, crystals grown under different crystallization conditions can be inspected sequentially in a high throughput manner. Indeed, the method can be automated so that large numbers of crystallization conditions can be examined with minimal expense. In addition, the method and apparatus can easily be used to optimize the X-ray diffraction quality of crystals in addition to optimization of their size and visual appearance.

According to the method of the invention, a typical crystallization experiment is analyzed for crystal growth by passing an X-ray beam through a crystallization drop and assessing, through the use of a detector, whether any crystals in the drop diffract

the X-rays. Diffraction indicates that a crystal has successfully grown. If no diffraction is observed, the experiment is either allowed to incubate further or ruled a failure. Moreover, the quality of the diffraction pattern may be assessed to determine the quality of the crystal and thereby optimize crystallization conditions.

Even for a small crystal or a microcrystal, the method and apparatus of the present invention provide an indication of the diffraction quality of the crystal and ideally the crystals resolution limit. Furthermore, the method and apparatus of the present invention can be used to distinguish amorphous precipitate from microcrystals, where a powder diffraction pattern of an X-ray beam by a sample is indicative of ordered microcrystals. In fact, a powder diffraction pattern produced by the method and apparatus of the present invention can even be used to assess the diffraction quality of the microcrystals.

The method and apparatus of the invention can further be used to differentiate between a protein crystal and a non-protein crystal, such as, for example, a salt crystal. This distinction may be made from, for example, analyzing the size of the crystal lattice.

As a result, the apparatus and method of the invention not only permit a determination of whether any crystal growth has taken place, by virtue of the ability to observe the quality of a diffraction pattern, but they also permit several different crystallization experiments to be compared to one another in an effort to identify optimal crystallization conditions.

The apparatus and method of the invention may be used for virtually any crystallization process known to those of skill in the art, including, but not limited to, hanging drop, sitting drop, microbatch, dialysis and gel crystallization. Moreover, the method may be readily automated, permitting the high throughput discovery of optimal crystallization conditions with minimal user input.

Accordingly, the invention provides an apparatus for detecting the presence of crystals in their *in-situ* growth environment. The apparatus comprises a crystal growing incubator having opposing first and second sides. The apparatus further includes an X-ray system that comprises an X-ray source disposed adjacent to the first side of the crystal growing incubator, and an X-ray detector disposed adjacent to the

second side of the crystal growing incubator. The X-ray source is configured to irradiate crystals grown in the crystal growing incubator and the X-ray detector is configured to detect the presence of diffracted X-rays from crystals grown in the crystal growing incubator. The apparatus preferably further comprises a positioner that positions the incubator and the X-ray system relative to each other. An imaging system, such as an optical imaging system, is preferably disposed adjacent to the crystal growing incubator to first detect the presence and location of potential crystals grown in the incubator.

Still further, a method of screening for crystals in their *in-situ* growth environment is also provided. Once a potential crystal has been grown in a crystal growing incubator the crystal growing incubator is preferably coupled to a positioner. Preferably, the presence and/or location of the potential crystal in the crystal growing incubator is then optically determined using the imaging system. The location is optionally stored, and the crystal growing incubator and X-ray system are accurately aligned relative to each another based on the location of the potential crystal to ensure that an X-ray beam emitted from the X-ray source is accurately directed at the potential crystal. The potential crystal is then irradiated with the X-ray beam. If the X-ray detector detects the presence of a diffraction pattern from the potential crystal, a crystal is thereby identified and can then be screened and/or optimized for diffraction quality. In this way, potential crystals grown in the incubator can be screened for suitability by the X-ray system, thereby facilitating the increased reproducibility of successful crystal growth experiments.

Further, the apparatus and method may be used in various environments such as, for example, on earth or in space, such as, for example, in a space station or a spacecraft. An advantage of the using the present invention in space is that crystal growth can be monitored remotely. Further, remote monitoring of crystal growth may be an advantage, for example, for monitoring toxic proteins such as, for example, virus particles or bacterial toxins.

BRIEF DESCRIPTION OF THE DRAWINGS

For a better understanding of the invention, reference should be made to the following detailed description, taken in conjunction with the accompanying drawings, in which:

FIG. 1 is a diagrammatic side view of an apparatus for screening for crystals according to an embodiment of the invention, where the apparatus is shown in partial cross-section for explanatory purposes;

FIG. 2 is a diagrammatic side view of an imaging system according to another embodiment of the invention, where the apparatus is shown in partial cross-section for explanatory purposes;

FIG. 3A is a top view of a positioner according to an embodiment of the invention;

FIG. 3B is a side view of the positioner shown in **FIG. 3A**, where the apparatus is shown in partial cross-section for explanatory purposes;

FIG. 4 is a flow chart of a method of screening for protein crystals according to an embodiment of the invention.

FIG. 5 is a perspective view of an another embodiment used in an experiment according to the invention; and

FIG. 6 is a X-ray diffraction image obtained using the embodiment of the invention shown in **FIG. 5**.

Like reference numerals refer to corresponding parts throughout the several views of the drawings.

DETAILED DESCRIPTION OF THE INVENTION

According to the invention, the diffraction quality of a crystal or a candidate crystal can be efficiently evaluated without disturbing the crystal from its crystallization solution, i.e., growth environment. A crystallization solution can thus be screened *in-situ* to determine whether or not crystal growth has taken place. Where the crystallization solution is not disturbed from its crystallization environment, the crystallization solution can further incubate after an initial screen and then be screened at a later time. The crystallization solution can even be screened multiple times.

Furthermore, multiple crystallization solutions can be rapidly and sequentially screened for crystal growth in a high throughput manner.

Screening can include the identification of crystalline material in one or more crystallization solutions. Screening can also include comparison of the diffraction quality of a number of crystals in a number of crystallization solutions. Such comparison can be used to, for instance, optimize the diffraction quality of crystals by assaying a number of crystallization solutions. In some embodiments of the invention, screening may also include both the identification of crystals and the comparison or optimization of diffraction quality.

The method and apparatus of the invention can be used to screen for crystals of any type of molecule. For instance, the method and apparatus of the invention can be used to screen for crystals of small molecules or macromolecules or other molecular crystals known to those of skill in the art. Suitable small molecules for the method and apparatus of the invention include, for example, small organic molecules, drugs, therapeutic molecules, antibiotic molecules, antiviral molecules, peptides, amino acids, oligonucleotides, nucleotides, sugars and other small molecules known to those of skill in the art. Suitable macromolecules include, for example, proteins, polypeptides, antibodies, enzymes, nucleic acid binding proteins, polynucleotides, DNAs, RNAs, carbohydrates and other macromolecules known to those of skill in the art.

The method and apparatus of the present invention can be used to screen crystals grown by any method of growing crystals known to those of skill in the art including, for instance, the vapor diffusion method, the hanging-drop method, the sitting drop method, the dialysis method, the microbatch method, and the gel crystal growth method. For example, native crystals can be grown by dissolving a substantially pure molecule in a crystallization solution containing a precipitant at a concentration just below that necessary to precipitate the molecule. Water can be removed from the crystallization solution by controlled evaporation to produce precipitating conditions, which are maintained until crystal growth ceases.

In one embodiment, native crystals are grown by vapor diffusion in hanging drops (McPherson, 1982, Preparation and Analysis of Protein Crystals, John Wiley,

New York; McPherson, 1990, Eur. J. Biochem. 189:1-23.). In this method, the molecule and the crystallization solution are allowed to equilibrate in a closed container with a larger aqueous reservoir having a precipitation solution containing a precipitant at a concentration optimal for producing crystals. The crystallization solution is suspended as a droplet underneath a coverslip, which is sealed onto the top of the reservoir. The sealed container is allowed to stand until crystals grow.

A beam of X-rays is then passed through a crystallization solution to determine whether the solution contains crystalline material and/or to determine the diffraction quality of the crystalline material within the solution. For instance, if a solution contains a single, well-ordered crystal, or a few well-ordered crystals, a pattern of X-ray diffraction spots can be detected. If a solution contains randomly-oriented microcrystals, a powder diffraction pattern might be detected. Such a powder diffraction pattern generated by the *in-situ* method and apparatus of the present invention can even be used to characterize the diffraction quality of the microcrystalline material. The diffraction pattern or powder diffraction pattern correlates with the structure of the molecules comprising the microcrystals. If the X-ray beam passes through no crystalline material or if the beam passes through amorphous precipitate, no diffraction is observed.

Thus, in a method of the invention, a drop that may contain a crystal, or microcrystals, may be scanned by the X-ray beam. Those of ordinary skill in the art recognize that by using such a method, a powder diffraction pattern may indicate the presence of microcrystals or crystals.

Figure 1 is a diagrammatic side view of an apparatus **100** for screening for crystals according to an embodiment of the invention. The apparatus **100** principally comprises an incubator **102** (shown in partial cross-section for explanatory purposes), and an X-ray system comprising an X-ray source **114** and an X-ray detector **116**. The X-ray system is preferably capable of resolving 1.5 Å to 3 Å.

The incubator **102**, as used herein, is any apparatus in which crystals **108** can be grown. For example, the incubator **102** may be a crystallization tray or plate. The incubator thus provides an *in-situ* growth environment for a crystal **108**. For explanatory purposes, the incubator **102** shown in **Figure 1** may be used for growing

crystals using both a hanging drop **128** and a sitting drop **130** configuration, although in use, typically only one type of configuration per incubator **102** is used. Any suitable incubator **102** for growing crystals may be used, such as those disclosed in U.S. Patent Nos. 5,096,676 to McPherson, or 5,130,105 to Carter *et al.*, which are both incorporated herein by reference. The incubator **102** preferably comprises at least a lower or first side **110** and an opposing upper or second side **112**. The incubator **102** preferably also comprises a number of wells **122** configured to hold a precipitation solution **106** for vapor diffusion. In the hanging drop configuration **128**, a drop **104** of crystallization solution is applied to a glass cover slip (preferably coincident with the second side **112**) and placed upside down on top of each well **122**. These conditions lead to supersaturation in the drop **104** and the initiation of precipitation that forms a crystal **108**.

In the sitting drop configuration **130**, a drop **132** of crystallization solution is placed in a receptacle on the top of an upstanding column **126** where conditions lead to supersaturation in the drop **128** and the initiation of precipitation which forms a crystal **108**.

The X-ray source **114** is preferably disposed adjacent to the first side **110** of the incubator **102**, and the X-ray detector **116** is preferably disposed adjacent to the second side **112** of the incubator **102**. Alternatively, the positions of the X-ray source **114** and the X-ray detector **116** may be switched, such that the X-ray detector is disposed adjacent the first side **110** and the X-ray source is disposed adjacent the second side **112**.

The X-ray source **114** preferably consists of a Copper (Cu) target micro-focus X-ray tube that generates an X-ray flux of at least 5×10^8 photons/s/mm.[2] This X-ray flux is necessary in order to record a diffraction pattern from the crystal **108** while it is located within its *in-situ* growth environment within the crystallization experiment.

In a preferred embodiment, an X-ray beam **120** is emitted upward and perpendicular to the incubator **102**. The X-ray beam **120** emitted from the X-ray source **114** is preferably monochromatic and consists of CuKα radiation. The X-ray beam **120** can be of any wavelength and is preferably between about 0.5 Å and about 2.0 Å. Furthermore, the X-ray beam **120** is also preferably tightly focused and

collimated to minimize any X-ray scatter that might occur as a result of X-rays reflecting from the apparatus **100**. To form the tightly focused X-ray beam **120**, one or more mirrors and/or collimators **118** may be provided. The collimators **118** are preferably disk shaped with circular holes extending there-through. To further aid in the accurate collection of diffracted X-rays from the crystal **108**, the X-ray beam **120** is preferably less than or equal to the size of the crystal **108** being irradiated. As even the largest crystals grown for routine X-ray structure determination are generally less that 0.5 mm in size in their largest dimension, and more routinely are around 100-200 microns, the X-ray beam **120** preferably has a focus size of 200 microns or less.

In one embodiment, the X-ray source **114** is a BEDE MICRO-FOCUS (or MICROSOURCE) X-RAY GENERATOR manufactured by BEDE SCIENTIFIC INSTRUMENTS LIMITED. Micro-Mirror X-ray optics from BEDE or REFLEX are also preferably used. Alternatively, a suitable X-ray source **114** is a standard RIGAKU INTERNATIONAL CORP rotating anode generator.

Alternatively, in another lower cost embodiment, a micro-focus tube, such those made by KEVEX or FEINFOCUS, combined with a single or a dual-lens system using capillary optics from X-RAY OPTICAL SYSTEMS, and a Confocal MaxFlux multi-layer optics from OSMIC or RIGAKU, may be used. The capillary optics gather in a larger solid angle of X-rays from the source spot and the Confocal MaxFlux provides the wavelength selection and final collimation. For even lower costs, a single instead of dual focusing system can be used.

In yet another embodiment, a (non-rotating anode) mini-focus X-ray tube can be used to obtain more flux. The larger spot of the mini-focus tube at 200 watts provides a flux of 8 times that of a 25 watt microfocus tube from BEDE. In yet another embodiment, the beam size is preferably about 50 microns and the beam spot is preferably about 40 microns in diameter. In yet another embodiment, a synchrotron beam may be used.

The incident X-ray beam **120** has greater penetration than any scattered X-rays, and thus, in a vapor diffusion embodiment, the apparatus **100** is preferably set up such that the X-ray beam **120** passes through the bottom or first side **110** of the incubator **102**, and through the precipitant **106** in the well **122**. The X-ray beam **120**

is then diffracted by any crystals **108** in its path. In this embodiment, diffracted X-rays **124** from crystal **108** pass through the upper or second side **112** of the incubator **102** and through an air gap to the detector **116**, virtually unimpeded. In fact, the X-ray beam preferably can penetrate up to 1.5 mm of polystyrene and/or up to 5 mm of oil.

In the hanging drop configuration **128** the upper or second side **112** is typically a glass cover slip or mylar tape. In a microbatch embodiment under oil, the orientation of the X-ray beam relative to the incubator **102** is not critical. In the microbatch embodiment, the X-ray detector can be located adjacent the second side **112** of the incubator, i.e., above the incubator **102**, and the X-ray beam could pass through a covering layer of oil and be detected by the detector **116** adjacent the first side **110** of the incubator **102**, i.e., below the incubator.

The X-ray detector **116** is chosen for its sensitivity and speed and is preferably a two-dimensional detector that is sensitive to X-rays diffracted from a candidate crystal that pass through a planar surface. If a sample potentially contains microcrystals that might generate a powder diffraction pattern, the detector can be a one-dimensional or two-dimensional detector that records the position and intensity of diffracted X-rays from a candidate crystal. An ideal X-ray detector **116** preferably combines the high sensitivity of the phosphor plates with the rapid readout of a CCD camera.

Unlike other crystallography X-ray detectors, the X-ray detector **116** does not need to be large, as it does not need to resolve individual diffracted beams, but rather needs to observe the resolution limit of diffraction. Thus, a CCD camera would not require the demagnification glass taper which is needed for CCD detectors currently in use on X-ray sources. In addition, a one dimensional detector is adequate for detecting the resolution limit of diffraction, especially the resolution limit of a powder pattern.

A suitable X-ray detector should be at least as sensitive as the presently commercially available imaging plate systems, such as the phosphor plates made by FUJIFILM MEDICAL SYSTEMS U.S.A., INC (for example, the BAS 2500 NDT) which are some of the most sensitive X-ray detectors that currently exist.

However, in a preferred embodiment, the X-ray detector **116** is a cooled Charge Coupled Device (CCD) camera, which although being slightly less sensitive to X-rays than available imaging plate systems, has a very rapid readout time. Such a CCD detector is preferably mounted on a swing axis that has an imaginary center on the crystal and rotates so that the detector's input faceplate is perpendicular to the X-ray beam and can swing up to about 50 degrees from perpendicular. The CCD detector preferably includes a selected phosphor screen, with or without a fiber-optic taper front-end. The phosphor preferably achieves 4 to 8 line-pairs per millimeter resolution. In a preferred embodiment, the CCD detector is placed about 80 mm from the crystal.

In a preferred embodiment, a beam-stop **126** is provided between the incubator **102** and the X-ray detector **116** to stop a central non-diffracted X-ray beam from damaging the detector or adversely affecting the results. The spot size of the X-ray beam **120** at the detector (or beam stop) is preferably about 40 to 50 microns in diameter, with a divergence of no greater than 30 arc-seconds. A means for inserting a calibration crystal into the X-ray beam, is also preferably provided to calibrate the apparatus **100**.

Figure 2 is a diagrammatic side view **200** of an imaging system according to an embodiment of the invention, where the incubator is shown in partial cross-section for explanatory purposes. Due to the size of the tightly focused X-ray beam **120** **(Figure 1)**, the X-ray beam and the crystal **108** **(Figure 1)** must align precisely in order for the crystal to precisely diffract the X-ray beam. For the hanging drop configuration **128** **(Figure 1)**, a crystallization drop of about 2 μL forms a hanging drop of about 1 - 2 mm in diameter. As mentioned above, the X-ray beam preferably has a focus size of 200 microns or less. Being that a potential crystal could be found at any position within this drop, the tightly focused X-ray beam **120** must be precisely aligned to irradiate the potential crystal. Although the utmost care is taken during setup procedures to position the drop **212** along a central axis **214** passing through the center of each well **122**, the drop may shift at some time prior to irradiation, to an off-center position **208**. What is more, many drops do not form sufficient crystals and only form amorphous precipitate **210**. To assist in aligning the X-ray beam with a

crystal, the present invention preferably employs an imaging system **202**. This imaging system **202** determines whether a crystal is present in a particular well **122**, and if so, determines and stores the precise location of the crystal for later alignment with the X-ray beam.

In a preferred embodiment, the X-ray system **114** and **116** (**Figure 1**) is coupled to the imaging system **202**. The imaging system **202** permits a rapid scan of a crystallization solution **104** (**Figure 1**) for potential crystals so that each potential crystal can be identified and its location stored for later alignment with the X-ray beam **120** (**Figure 1**). Use of imaging system **202**, reduces the exposure of a crystallization drop to X-rays, as the crystallization drop need not be exposed to an X-ray beam until a potential crystal has been located. Further, the imaging system **202** significantly reduces the time that each crystallization solutions needs to be exposed to the X-ray beam, thereby increasing overall throughput. This is particularly advantageous, as many molecules, such as proteins and other macromolecules are sensitive to irradiation by the X-ray beam and some might even denature in an X-ray beam.

Prior to aligning the crystal and the X-ray beam, the presence and/or location of each crystal in the incubator is first determined by the imaging system **202**. The presence and/or location is then stored. The stored location of each crystal is then used by a positioner (**Figure 3A** and **3B**) to align each crystal and the X-ray beam relative to one another. In the preferred embodiment, the imaging system is a video imaging system, where the image capture time is on the order of a second or less for each well **122**, as compared to what may be several minutes for the X-ray diffraction.

The X-ray system and the crystal preferably move relative to one another to ensure alignment of the X-ray beam and the crystal. In a preferred embodiment only the incubator **102** is moved relative to the X-ray system. In an alternative embodiment, the X-ray system, or both the X-ray system and the incubator **102** are moved relative to one another to align the crystal and the X-ray beam. This relative movement is undertaken by a positioner, which is discussed in further detail below in relation to **Figure 3A** and **3B**.

Figure 3A is a top view of a positioner **300** according to an embodiment of the invention. **Figure 3B** is a side view of the positioner **300** shown in **Figure 3A**, where the incubator is shown in partial cross-section for explanatory purposes. The incubator **102** is arranged on the positioner **300** which can move the incubator **102** along three perpendicular axes x, y, and z. A set of bushings **302** allow the incubator **102** to slide along parallel shafts **304** permitting translation of the incubator **102** along the x-axis. In a similar manner, bushings **306** allow the incubator **102** to slide along parallel shafts **308** permitting translation of the incubator **102** along the y-axis, while bushings **320** allow the incubator **102** to slide along parallel shafts **312** permitting translation of the incubator **102** along the z-axis. It should be appreciated that any suitable mechanism that translates the incubator **102** along any one of multiple axes may be substituted for the positioner **300** shown in **Figure 3A** and **3B**. For example, the positioner may rotate the incubator about an axis instead of linearly translating it. Furthermore, in order to accurately irradiate a crystal detected by the imaging system, the positioner **300** should be accurate to within a few microns.

Figure 4is a flow chart of a method of screening for crystals according to an embodiment of the invention. The incubator **102 (Figure 1)** is preferably first placed (step **402**) onto the positioner **300 (Figure 3)**. The incubator **102** can conveniently be placed with a "pick and place" robot arm known to those of skill in the art. The imaging system **202 (Figure 2)** is then preferably activated (step **404**) and moved over the incubator. Alternatively, the positioner can move the incubator relative to the imaging system. The activation (step **404**) of the imaging system entails scanning each well **122** to determine (step **418**) the presence and/or location (step **416**) of a potential crystal. The imaging system **202**, therefore, scans each well of the incubator for potential crystal material such as single crystals and microcrystals. The location of each visually acceptable potential crystal is then preferably stored (step **406**) by the imaging system. The imaging system is then retracted from its scanning position adjacent to the incubator. Using the stored location of each potential crystal, the positioner moves the incubator **102** or the X-ray detector **116** to align or position (step **408**) each potential crystal with a line coincident with the emitted X-ray beam. Each located potential crystal is then irradiated (step **410**) by the X-ray beam **120 (Figure**

1) emitted from the X-ray source **114** (**Figure 1**) . The X-ray detector **116** (**Figure 1**) detects (step **412**) any diffraction from the irradiated crystal, whereafter the detected diffraction patterns are stored and/or analyzed. The positioner can optionally locate the next potential crystal (step **414**) and the process can be optionally repeated until all detected crystals have been irradiated and their diffraction patterns stored and/or analyzed, where the diffraction patten indicates the presence of one or more well ordered crystal. The overall time to assess the quality of diffraction of a single crystal is approximately 5 minutes.

Furthermore, diffraction from a microcrystalline precipitate forms a powder pattern, whereas diffraction from an amorphous precipitate only forms diffuse scatter. Acceptable powder patterns indicate that microcrystals that have successfully formed. Therefore, the system screens (step **420**) for suitable crystals based on the diffraction patterns or powder patterns detected, where successful crystal growth is an indication of ideal growth conditions and can be used to refine any further crystal growth experiments.

Figure 5 is a perspective view of an another embodiment **500** used in an experiment according to the invention. A 1536 well plate **506** is secured to two mounting arms of an x-y translation device **502**. Plate **506** can advantageously be positioned at any angle so that the plate can be translated, with any suitable device, in the plane perpendicular to the X-ray beam **504**. The x-y translation device **502** can be used to position the plate **506** with respect to an X-ray beam **504** and a detector **510**. The plate **506** can be oriented with respect to the X-ray beam **504** so that a well **512** and any potential crystals within the well **512**, through which the X-ray beam **504** passes, can be identified. Diffracted X-rays are detected with detector **510**.

EXAMPLE 1

In this example, we describe the observation of X-ray diffraction from protein crystals *in-situ* using embodiment **500** of **Figure 5**. **Figure 6** is a positive X-ray diffraction image **600** obtained from one sample using the embodiment of the invention shown in **Figure 5**. Crystals of lysozyme were grown in wells **512** of a

plate **506** by the microbatch method, and X-ray diffraction to 1.8 Å resolution was observed by exposing the crystals in the plate **506** to an X-ray beam **504**.

The wells **512** in one corner of a Greiner 1536 well plate **506** were filled with paraffin oil (from HAMPTON RESEARCH). Each well was then injected with 400 nl of a 1:1 mixture of 60 mg/ml lysozyme in water (from SIGMA CHEMICAL CORP) and 6 - 10 % NaCl (Sigma) in 0.1 M sodium acetate buffer, pH 4.8. The plate **506** was spun gently to coax the aqueous drops of the 1:1 mixture to the bottom of each well **512**. After several days at room temperature (of about 20°C), crystals were visible in two wells **512** of the plate **506**.

Orientation of the plate **506** with respect to the X-ray beam **504** was first performed by visual alignment and then by X-ray diffraction from lead tape which was placed on the plate **506** to define an area of interest. One-second X-ray exposures were taken at 0.5mm intervals for each well **512** of interest. In this experiment, three wells were examined. Each well was exposed four times, once in each corner of the well. Diffraction spots from one of the three wells were observed, indicating that crystalline protein was present in the well.

All exposures led to a diffraction pattern that had a band of diffuse scattering **602** (**Figure 6**) which was centered around 4 - 5 Å resolution. This 4 - 5 Å band was probably due to diffraction from paraffin oil. On several, but not all, exposures, a second scattering ring centered around 8Å appeared. Since this second band appeared at exposures corresponding to approximately 2.5 mm translations of the plate, this ring was probably due to scattering from the walls of the wells of the plate which are spaced 2.25 mm apart. Some samples yielded no X-ray diffraction indicating that the X-ray beam did not pass through crystalline material. However, when the X-ray beam passed through some of the samples of lysozyme, intense diffraction patterns were observed indicating the presence of well-ordered crystals in the samples. Further exposures showed that one could observe diffraction out to 1.8Å from the lysozyme crystal *in-situ*. Examples of diffraction of the crystal are referenced by numeral **606**. It can also be observed that no diffraction occurs at the center **604** of the image due to placement of the beam stop **126** (**Figure 1**).

Various statistical indicators may be used to determine whether a diffracting crystal or microcrystal is present in a sample. The diffraction data may be analyzed extensively, but more preferably, a simple statistical analysis, such as detecting a standard deviation within the image, would be sufficient. One of ordinary skill in the art may, without undue experimentation, determine the threshold of standard deviation that would be appropriate to indicate the presence of crystals or microcrystals.

Thus, the method and apparatus of the present invention can be used to detect the presence of crystalline forms of molecules *in-situ*. In particular, determining whether a detected crystalline material is a protein crystal or a salt crystal. Furthermore, the resolution of the diffraction of crystalline material can be determined quantitatively.

Further, the apparatus and method may be used in various environments such as, for example, on earth or in space, such as, for example, in a space station or a spacecraft.

In this embodiment, a transmitter that transmits information associated with said diffraction pattern to a remote location is also provided. The transmitter may be any suitable transmitting means, such as radio, satellite, microwave, or the like. An advantage of the using the present invention in space is that crystal growth can be monitored remotely.

While the foregoing description, drawings and example represent the preferred embodiments of the present invention, it will be understood that various additions, modifications and substitutions may be made therein without departing from the spirit and scope of the present invention as defined in the accompanying claims. In particular, it will be clear to those skilled in the art that the present invention may be embodied in other specific forms, structures, arrangements, proportions, and with other elements, materials, and components, without departing from the spirit or essential characteristics thereof. The presently disclosed embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims, and not limited to the foregoing

description. All patents and publications disclosed herein are hereby incorporated by reference in their entirety.